Predictive Toxicology and In Vitro to In Vivo Extrapolation

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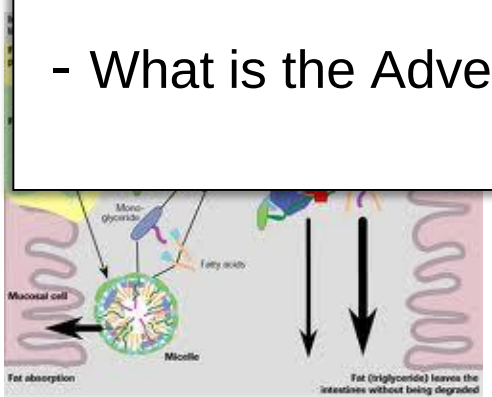
Problem Statement

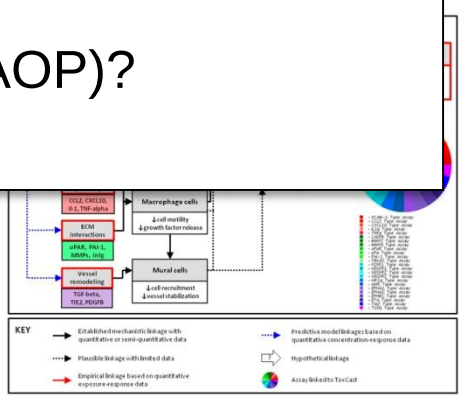
Too many chemicals to test with standard animal-based methods

- Cost, time, animal welfare

Need for better mechanistic data

- Determine human relevance
- What is the Adverse Outcome Pathway (AOP)?



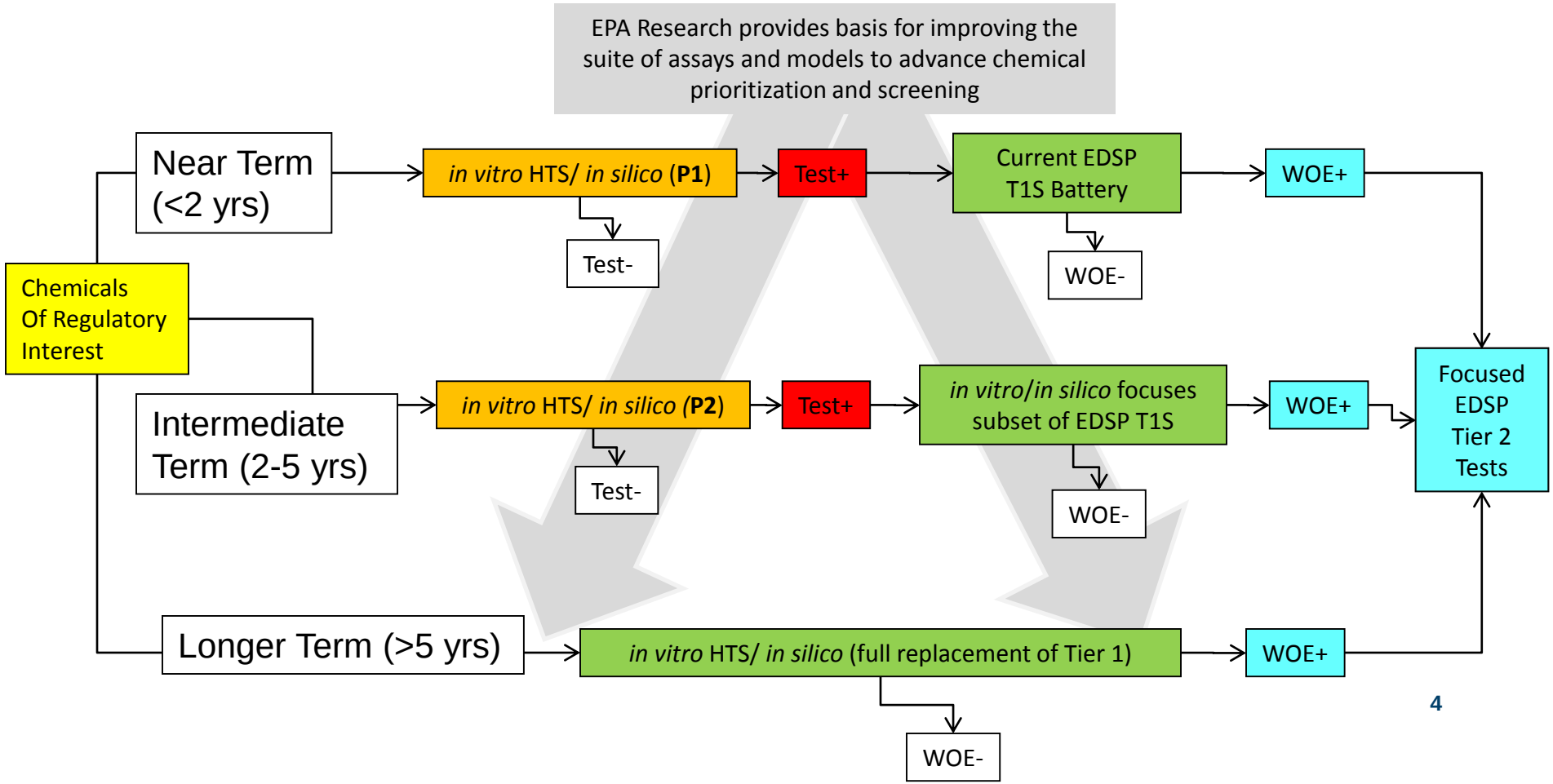


Computational Toxicology

- Identify biological pathways of toxicity (AOPs)
- Develop high-throughput *in vitro* assays to test chemicals
- Test “Human Exposure Universe” chemicals in the assays
- Develop models that link *in vitro* to *in vivo* hazard
- Use pharmacokinetic models to predict activating doses
- Develop exposure models for all chemicals

CompTox and the Endocrine Disruptor Screening Program

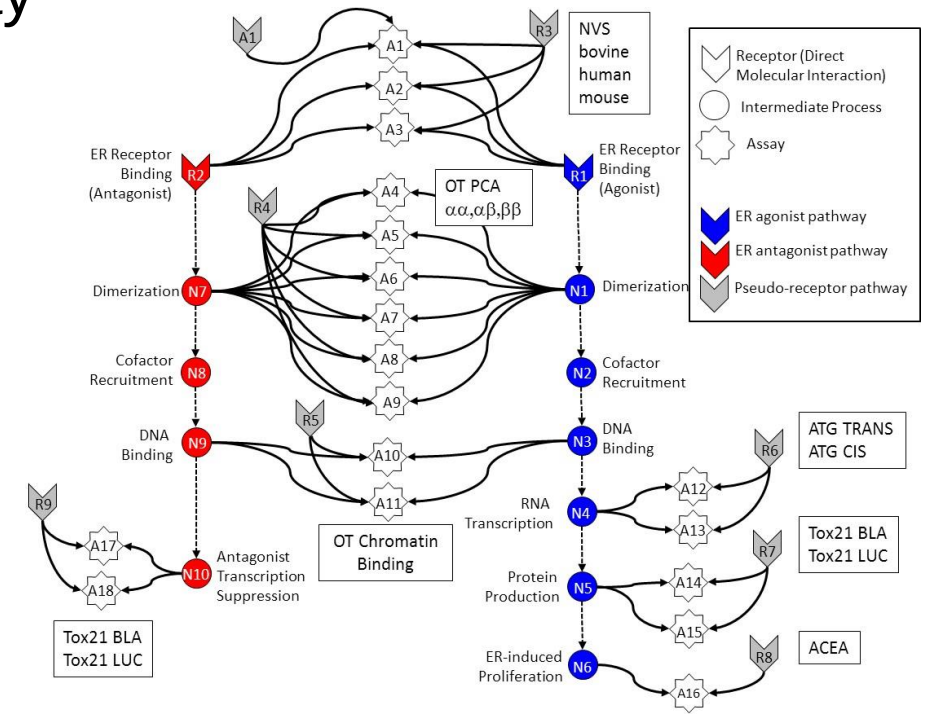
- 10,000 chemicals to be tested
- 100-200 years, $Billions of cost with current tests
- Need methods to prioritize chemicals
- Need high-throughput, lower cost replacement tests



In Vitro Estrogen Receptor Model

Combines results from multiple in vitro assays

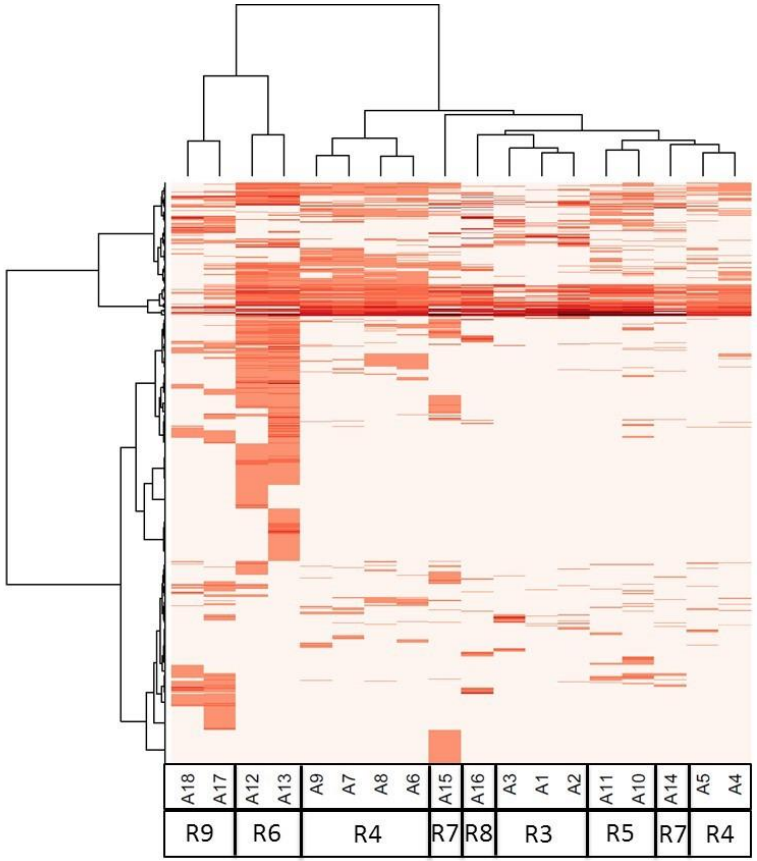
- Use multiple assays per pathway
  - Different technologies
  - Different points in pathway
- No assay is perfect
  - Assay Interference
  - Noise
- Use model to integrate assays
- Evaluate model against reference chemicals
- Methodology being applied to other pathways



Judson et al: "Integrated Model of Chemical Perturbations of a Biological Pathway Using 18 In Vitro High Throughput Screening Assays for the Estrogen Receptor" (submitted)

Major theme – all assays have false positives and negative

Assays cluster by technology, suggesting technology-specific non-ER bioactivity

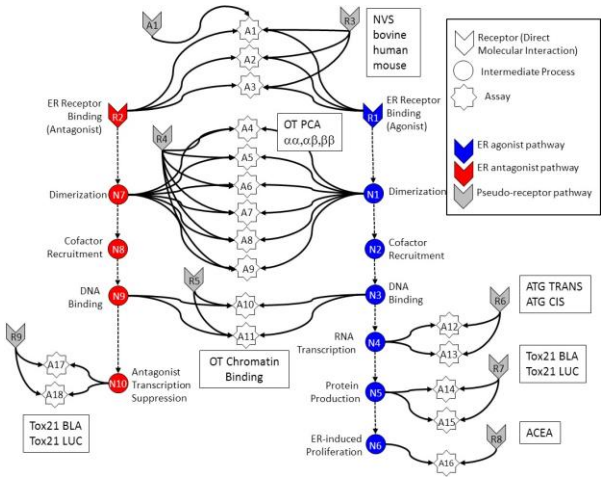


Much of this “noise” is reproducible

- “assay interference”
- Result of interaction of chemical with complex biology in the assay

EDSP chemical universe is structurally diverse

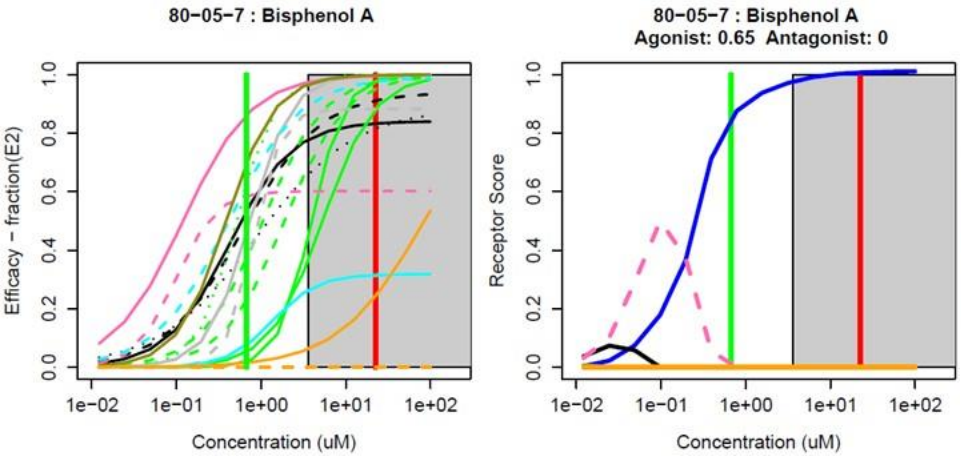
-Solvents
-Surfactants
-Intentionally cytotoxic compounds
-Metals
-Inorganics
-Pesticides
-Drugs



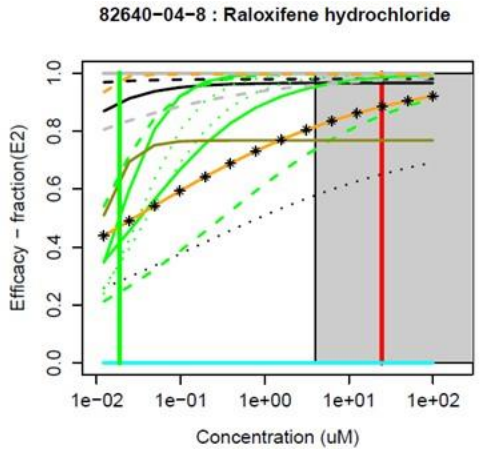
Example curves

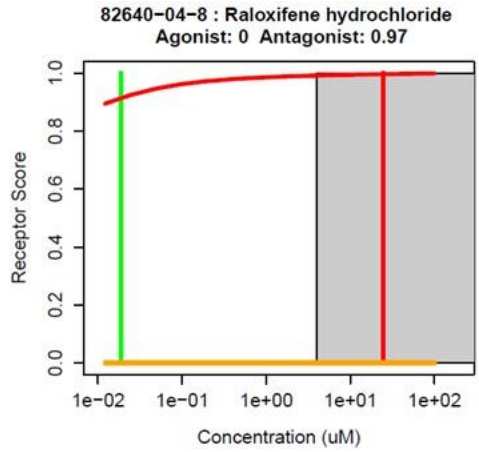
United States Environmental Protection Agency

True Agonist

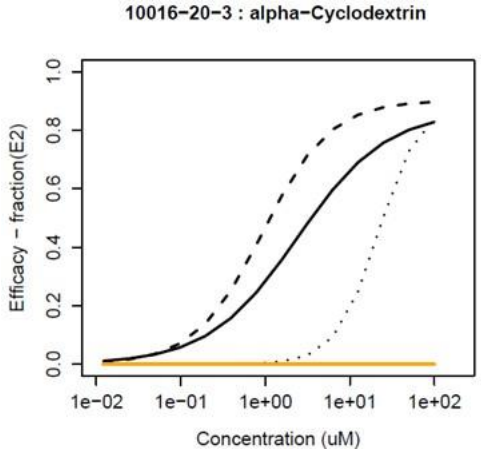


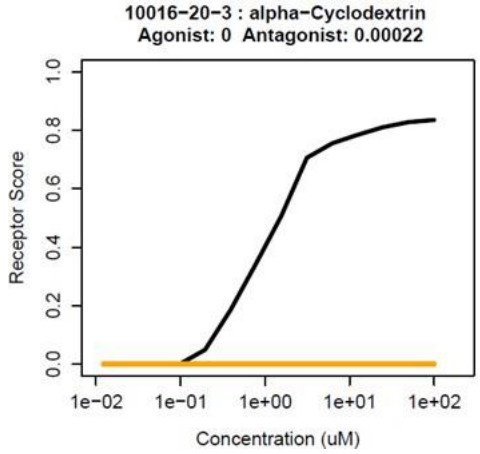
True Antagonist



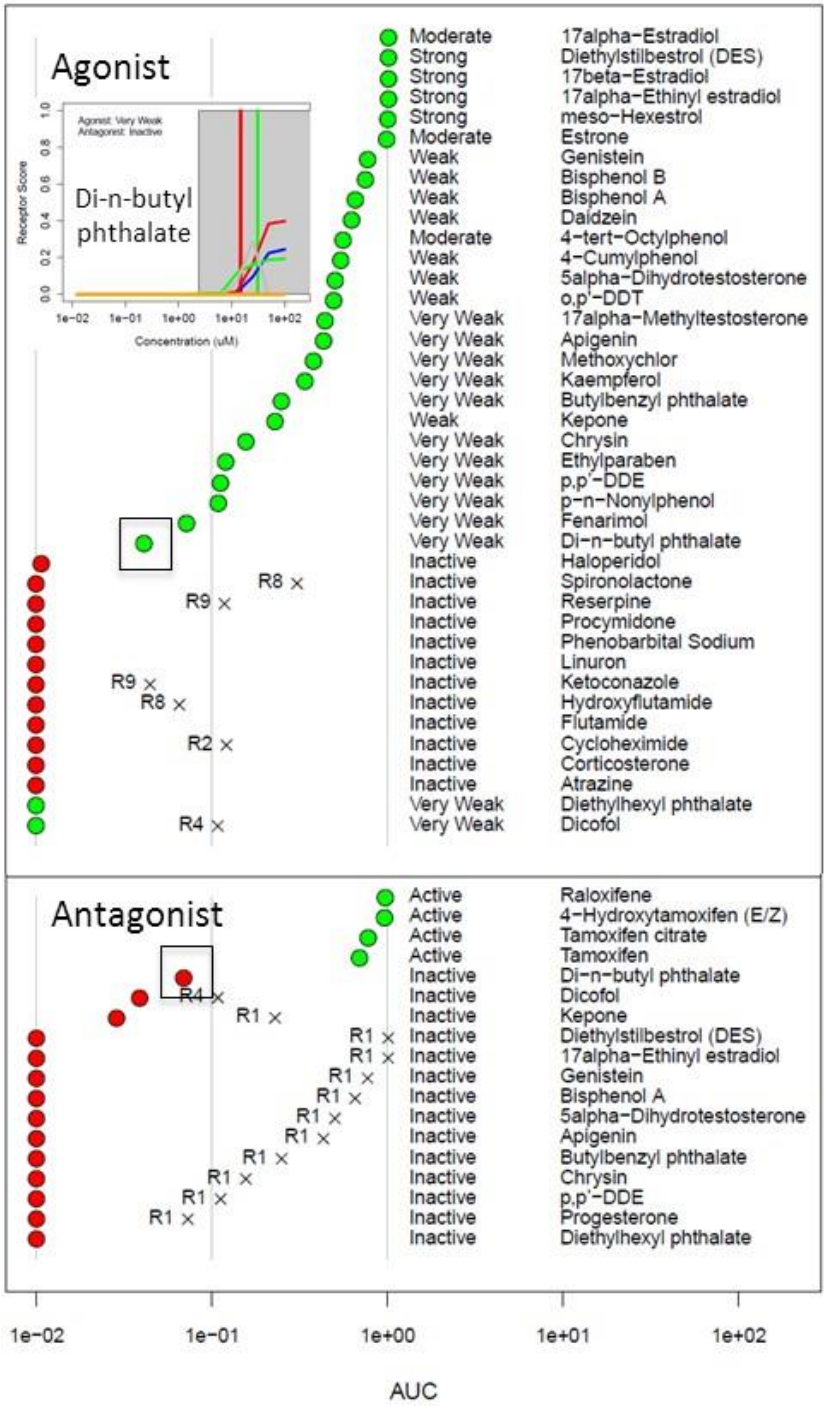


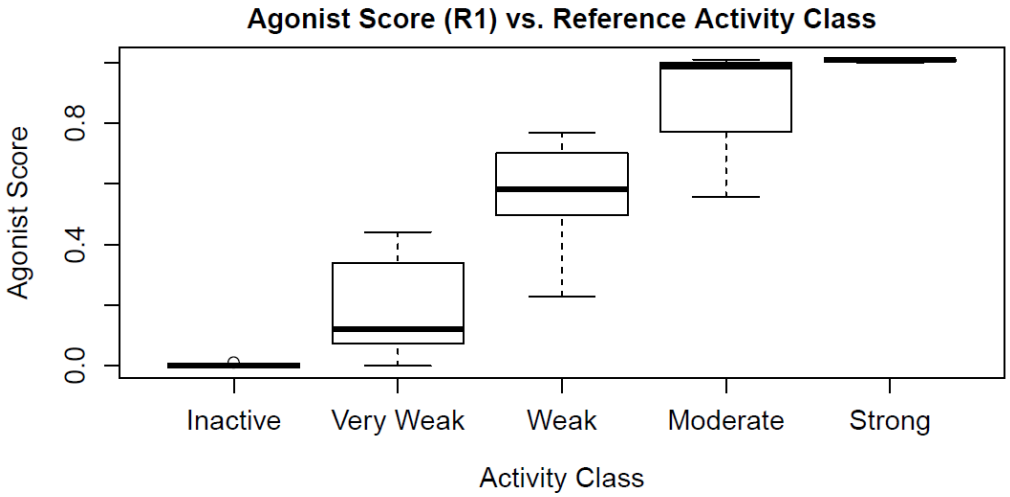
Negative-Narrow Assay Interference





In Vitro Reference Chemical Performance





In Vivo Reference Chemicals: Guideline Uterotrophic Assay Data

Uterotrophic Literature "Guideline-Like" Studies (start with 700 papers)

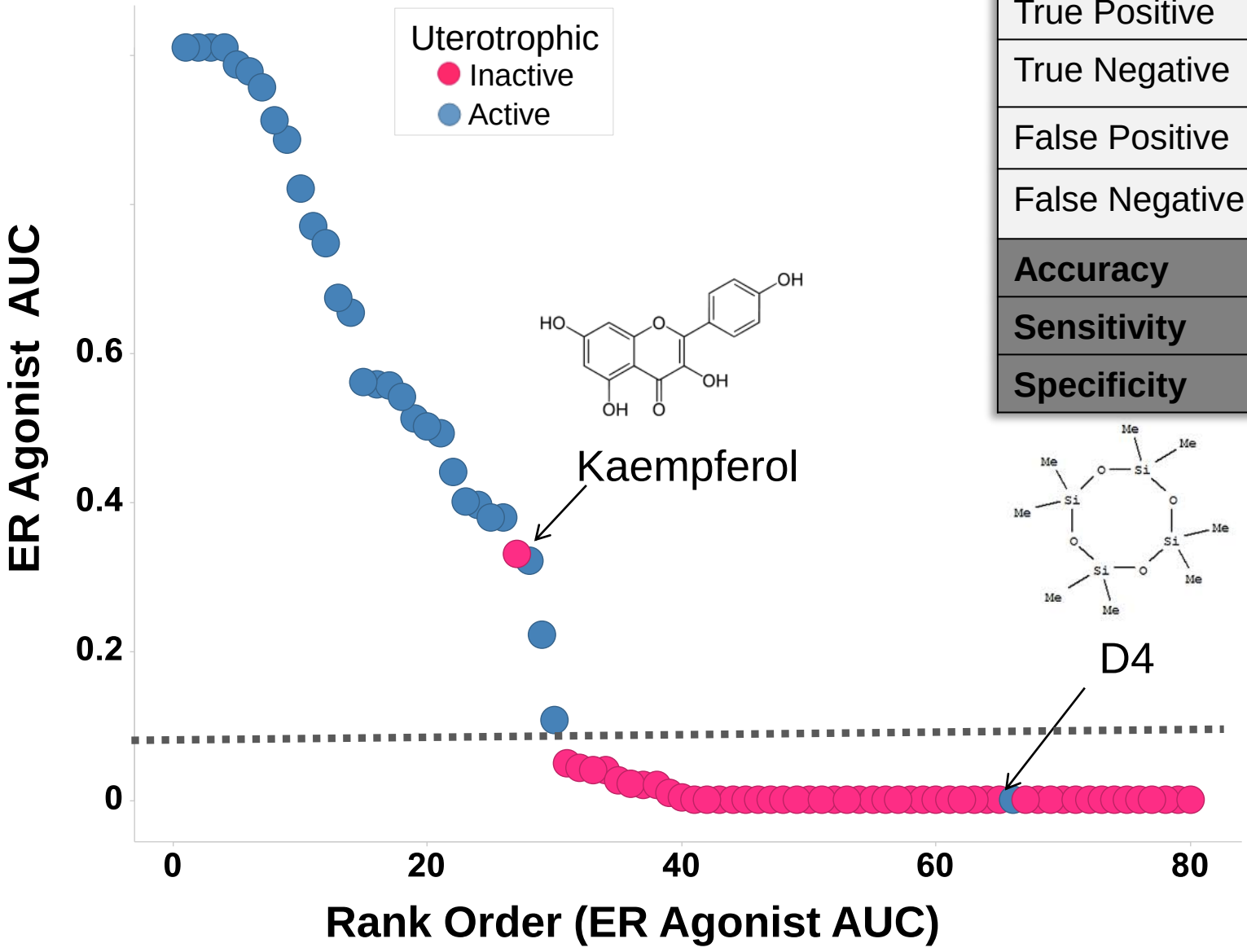
+

EDSP List 1 Uterotrophic "Guideline" Studies

Uterotrophic Reference Chemicals: 30 Active, 51 Inactive

Kleinstreuer et al: "A Curated Database of Rodent Uterotrophic Bioactivity" (submitted)

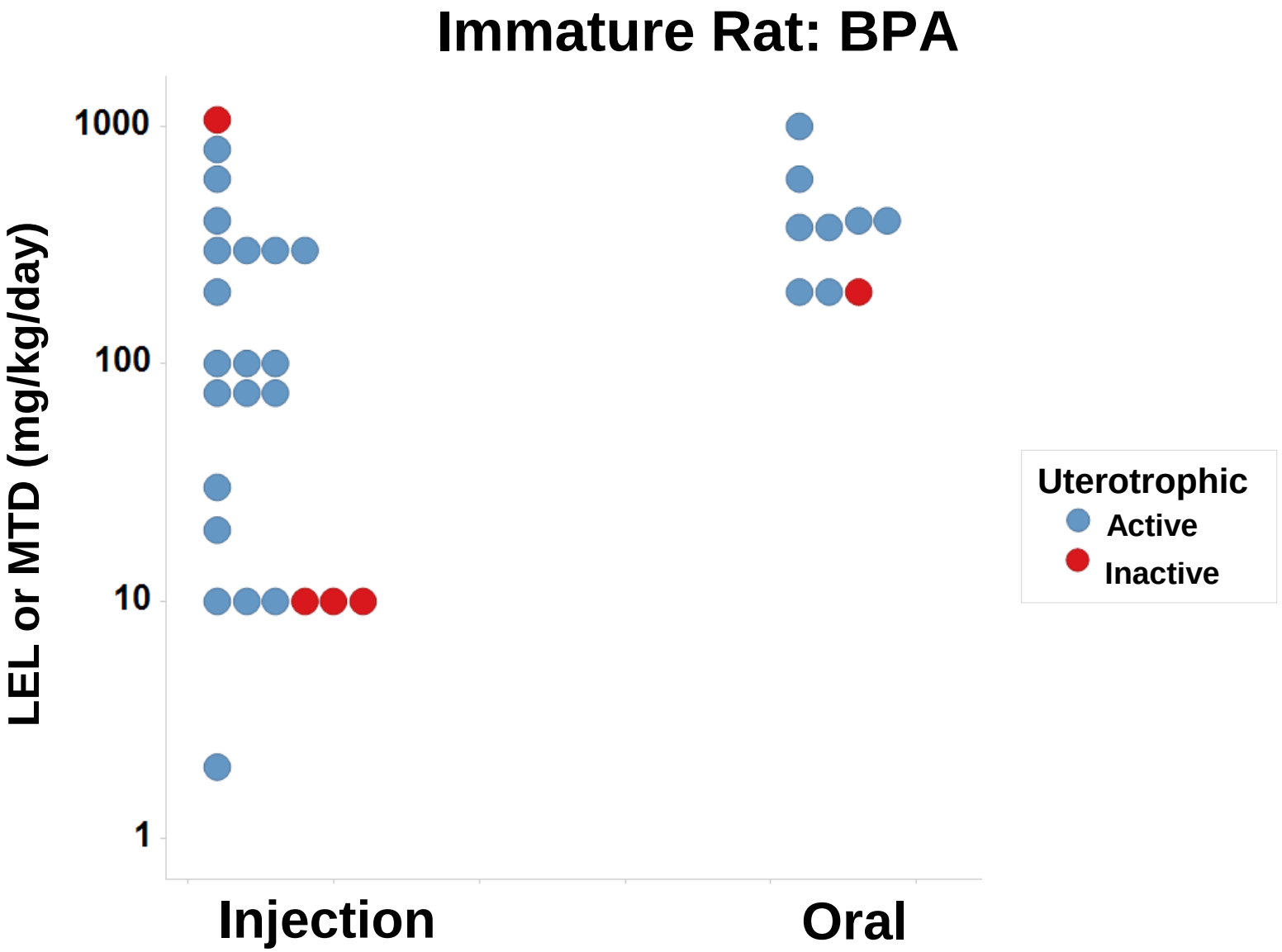
ER Agonist AUC vs. Uterotrophic Outcomes



True Positive	29
True Negative	50
False Positive	1
False Negative	1
Accuracy	**0.97**
Sensitivity	**0.97**
Specificity	**0.98**

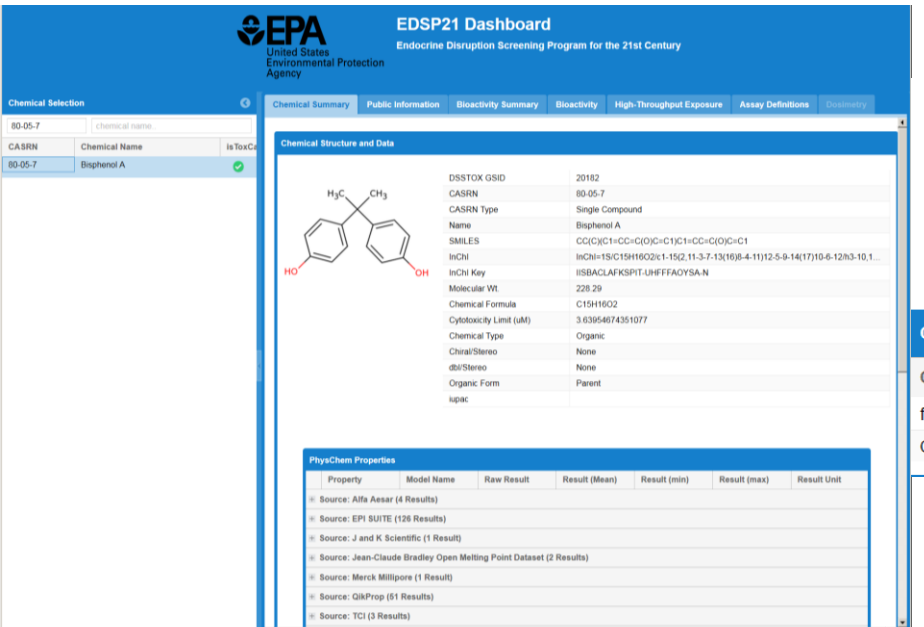
Browne et al. Screening chemicals for estrogen receptor bioactivity using a computational model (ES&T in press)

In vivo guideline studies have the same types of uncertainty as *in vitro*



Data Transparency: EDSP21 Dashboard

- Goal: To make ER and AR data easily available to all stakeholders
  - Assay-by-assays concentration-response plots
  - Model scores – AUC agonist and antagonist
  - ER QSAR calls
  - Other relevant data
- http://actor.epa.gov/edsp21



ToxCast Model Predictions		
Model	**Agonist AUC**	**Antagonist AUC**
ER	0.45	0
AR	0	0.136

Consensus CERAPP QSAR ER Model Predictions			
Class	**Agonist (Potency Level)**	**Antagonist (Potency Level)**	**Binding (Potency Level)**
from Literature	Active (Weak)	-	Active (Weak)
QSAR Consensus	Active (Weak)	Active (Strong)	Active (Weak)

Moving Towards Regulatory Acceptance From FIFRA SAP, December 2014

- Can the ER Model be used for prioritization?
  - "… the ER AUC appears to be an **appropriate tool for chemical prioritization** for … the EDSP universe compounds."

- Can the ER model substitute for the Tier 1 ER in vitro and uterotrophic assays?
  - "… **replacement of the Tier 1 *in vitro* ER endpoints …with the ER AUC model will likely be a more effective and sensitive measure for the occurrence of estrogenic activity** …"
  - "… the Panel **did not recommend that the uterotrophic assay be substituted** by the AUC model at this time. The Panel suggested that the EPA considers: 1) conducting limited uterotrophic and other Tier 1 in vivo assay testing, using the original Tier 1 Guidelines (and/or through literature curation)"

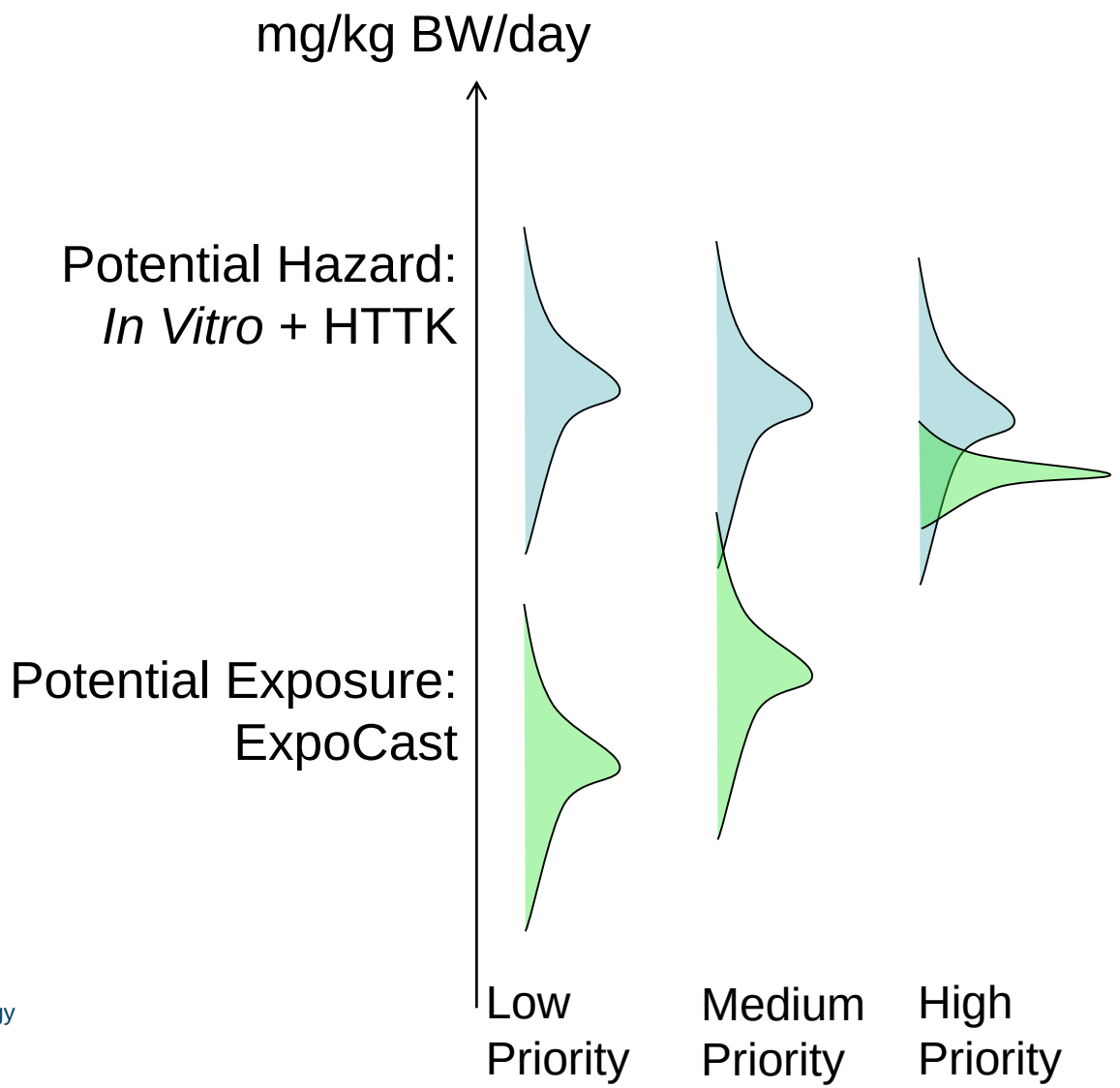
- Based on follow-up presented here (FR notice, June 18 2015) …
  - **"EPA concludes that ER Model data are sufficient to satisfy the Tier 1 ER binding, ERTA and uterotrophic assay requirements."**

Modeling Thyroid Disruption

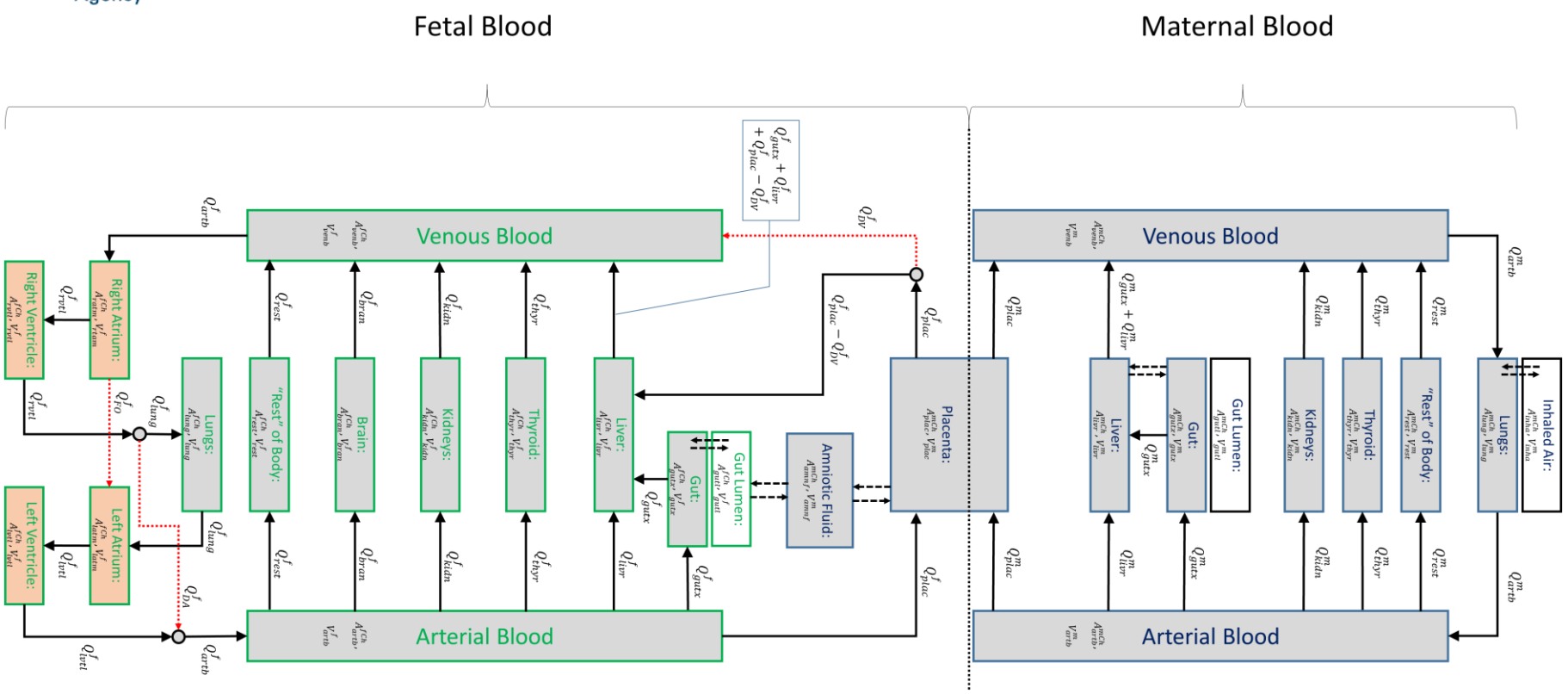
- Develop assays for key targets
  - Thyroid hormone receptor (Complete)
  - Thyroid peroxidase (TPO) (Screening in progress)
  - Deiodinases (assays in development)
  - NIS – Sodium-Iodide Symporter (assays in development)
  - Transporters (assays planned)
- Screen Chemicals
- Predict *in vivo* potency for assay hits
- Test effects in complex “tissue on a chip” systems

Risk-based Prioritization
Hazard + Exposure

Semi-quantitative *In Vitro* to *In Vivo* Approach



Maternal/Fetal PBPK Model



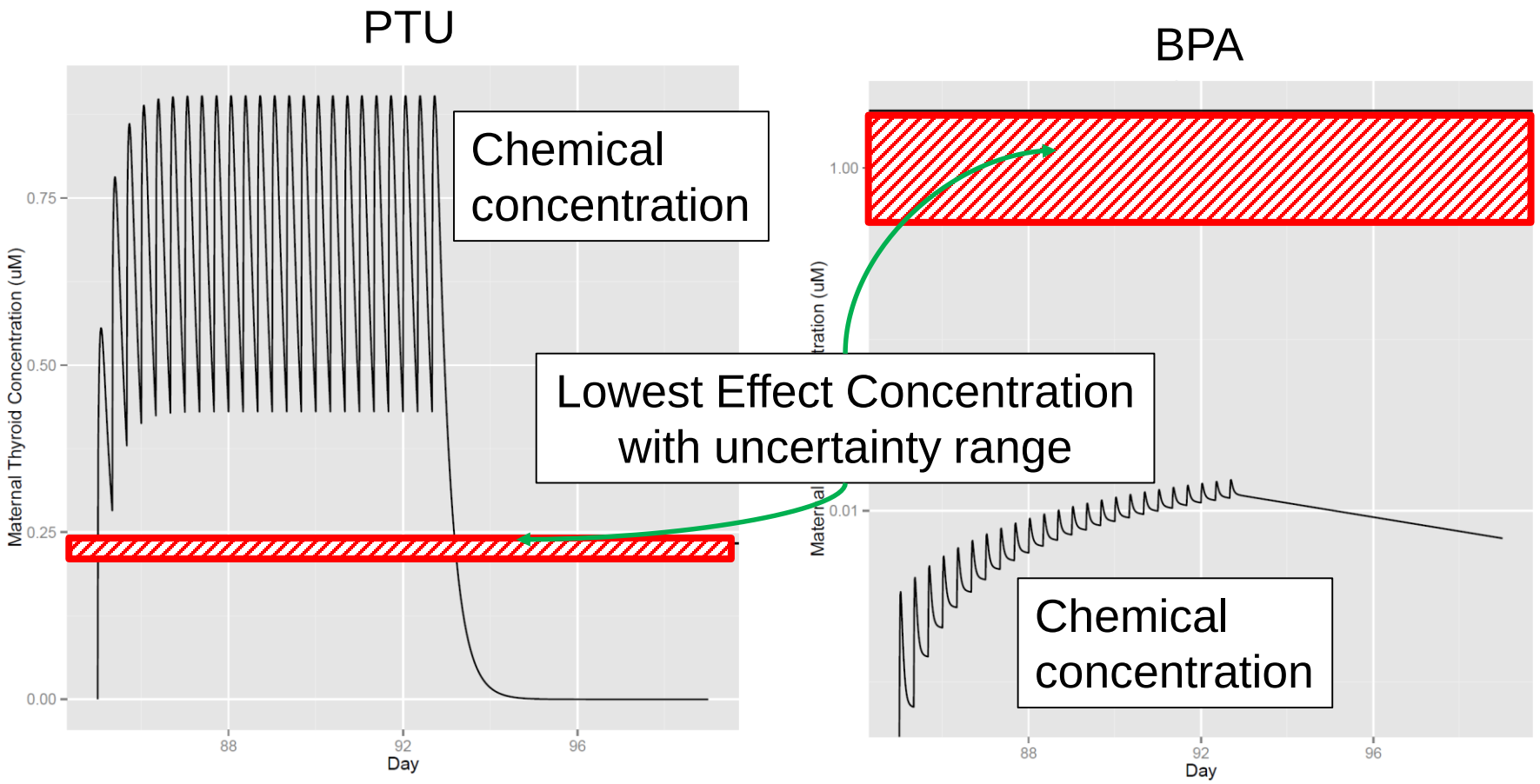
Model accounts for development of fetus, weeks 12 to term

Parameters for ~500 chemicals

Kapraun, Watt in prep

Prioritizing Chemicals Using the PBPK Model

PTU and BPA both target TPO (thyroid peroxidase)
But … effect of 1 mg/kg/day is much different

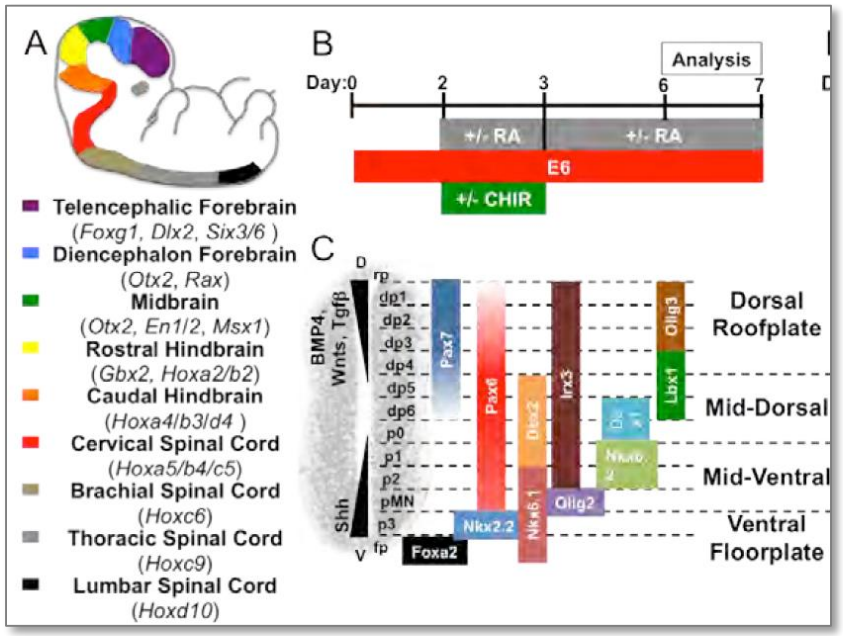


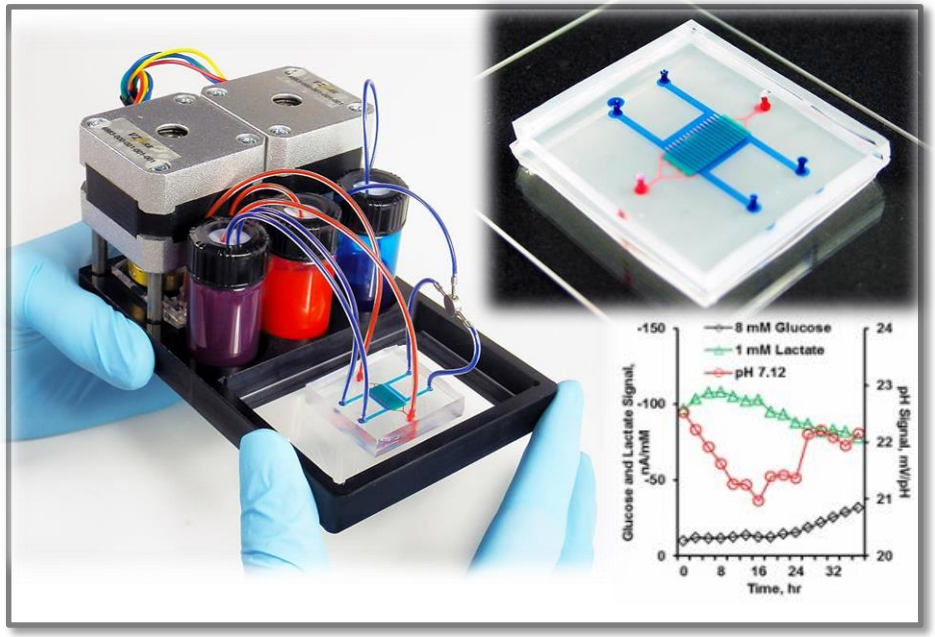
Kapraun, Watt in prep

Brain Development and the Neurovascular Unit

Randy Ashton (UWisc): 3D hCNS microsystem derived from hPSCs and patterned for phenotypic diversity across 9 discrete body axis domains.

John Wikswo (Vanderbilt): synthetic BBB (endothelia/pericyte/astrocyte) channel interfaced via porous matrix to neuron/microglia/WBC channel.

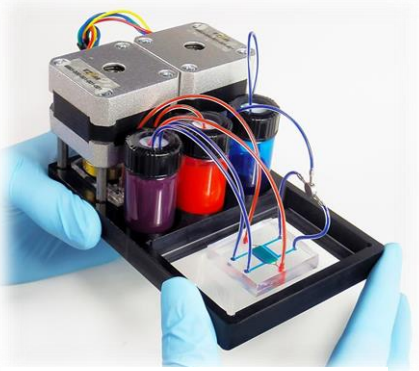




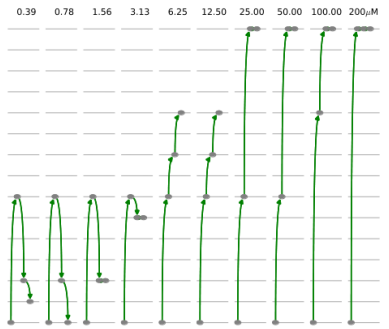
Collaboration to a synthetic model for thyrotropic neurodevelopment

Key Strengths and Weaknesses of *In Vitro* Systems for Toxicity Testing

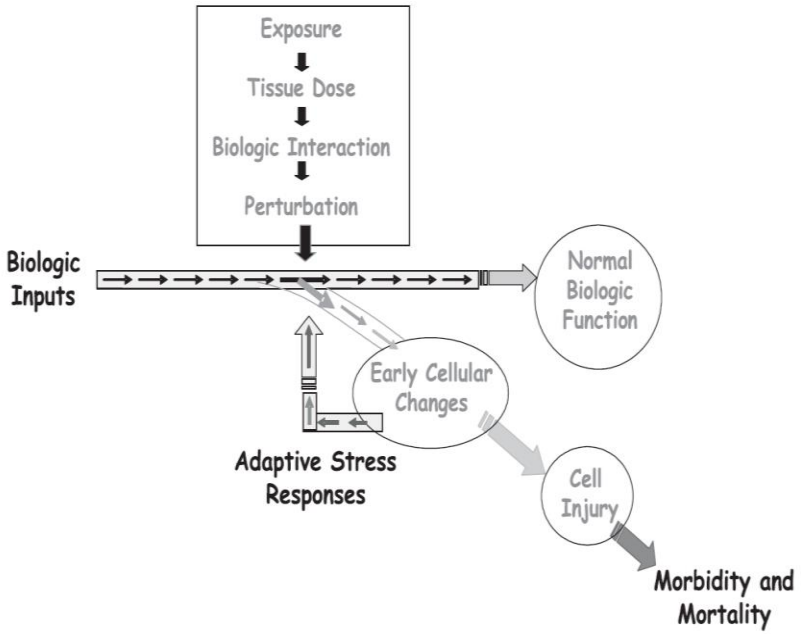
- Strengths
  - Rapid development of new assays
  - Ability to screen thousands of chemicals
  - Direct link to molecular basis of adversity



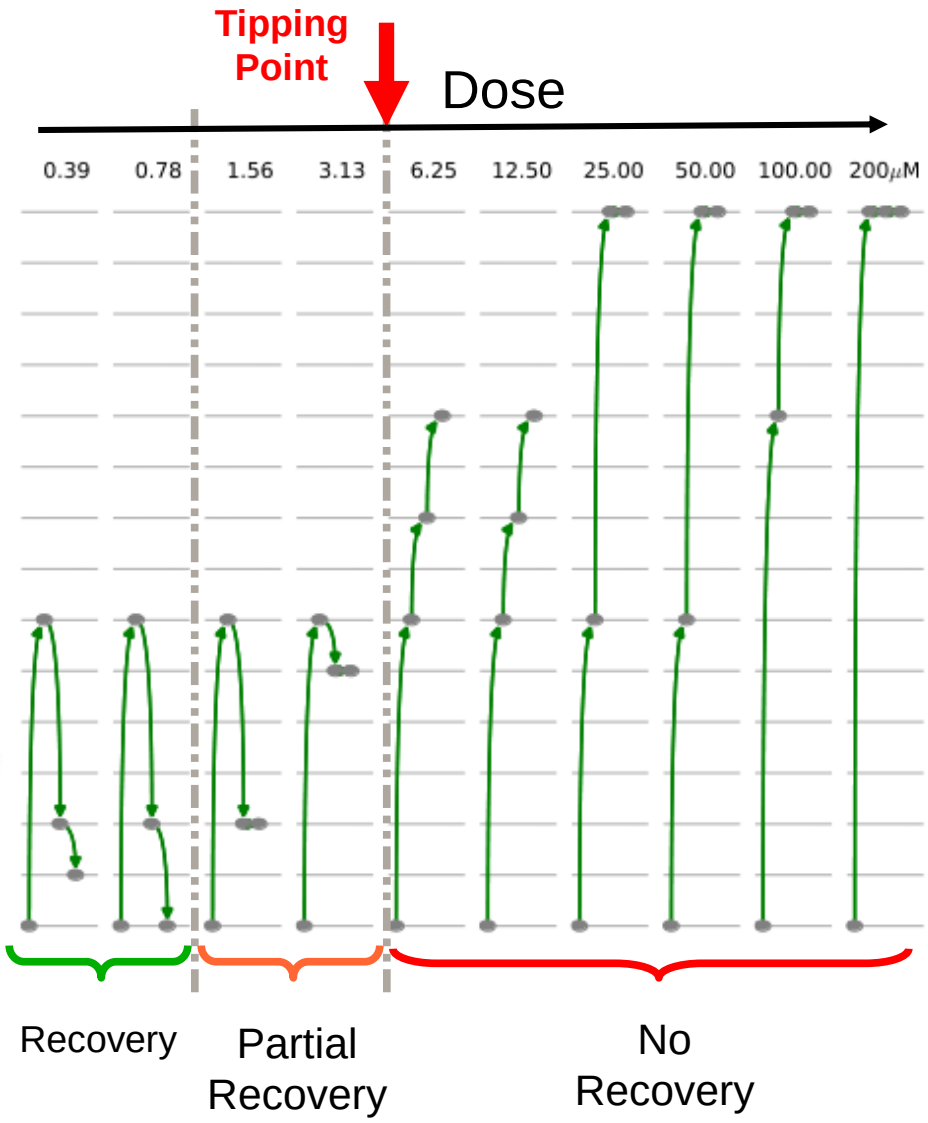
- Weaknesses
  - Often lack metabolic capacity
  - Often lack complex key multi-cell type signaling
  - Often lack ability to adapt



In Vitro Adaptation "Tipping Points" Use Time-Dose Trajectories



From NAS
Toxicity Testing in the 21st Century



Shah et al. in prep

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